



# A New High Content Screening Paradigm: Combination of Image Analysis Software and Microplate Cytometry

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## Abstract

Researchers are under increasing pressure to perform high content cell-based assays at throughputs compatible with primary screening. Where throughput is not an issue, microscope-based CCD imagers have predominated within the high content field, due to the breadth of biological assays that can be addressed by image analysis techniques. However, they have limited utility for screening due to their low throughput, limited field of view and generation of terabytes of image data.

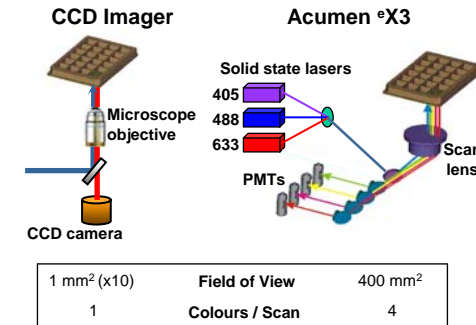
Laser-scanning fluorescence microplate cytometers combine the powerful object-recognition capabilities of CCD imagers with fast read speeds. An Acumen® X3 microplate cytometer (TTP LabTech, Melbourn, UK) performs cytometric analysis across entire wells at throughputs of up to 300,000 data points in 24 hours. Although regarded as low resolution devices, images generated from unprocessed photomultiplier tube readings correlate with those captured using a 20x microscope objective.

Acumen X3's enhanced software gives the flexibility of exporting whole well TIFF images (8 or 16-bit) for subsequent batch processing by third party image analysis software. The whole well imaging capability increases the utility of the Acumen X3 in areas where cytometric analysis is inappropriate, for example cell segmentation within monolayers. This new screening paradigm represents a major breakthrough in how microplate cytometers can be applied since rapid cytometric analysis can now be combined with image-processing methodology.

## Conclusion

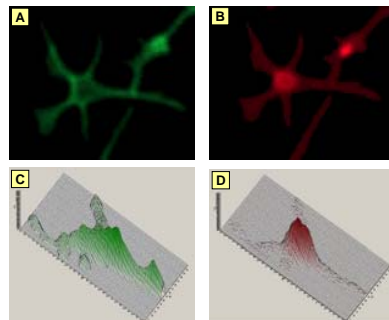
- Microplate cytometry is ideally suited for high-throughput, high-content screening
- Throughputs of > 300,000 well per day can be achieved without data storage issues
- Multiple-laser excitation offers similar wavelength range to that of white light source instrumentation for multiplexing
- TIFF image export extends the application of microplate cytometers into more detailed high content assays routinely requiring image processing.

## 1 Comparison of High Content Instrumentation Optics



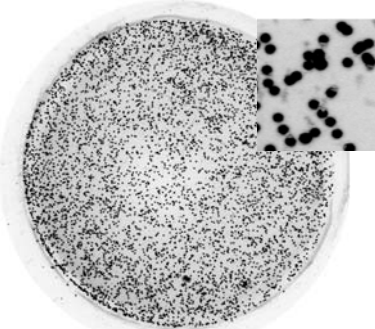
The large field of view (400 mm<sup>2</sup> (20 x 20 mm)) is far greater than that offered by microscope-based CCD imagers (~1 mm<sup>2</sup> for a x10 objective). The small field of view on microscope-based CCD imagers is only sufficient to obtain resolved images of around 100 cells at once. The application of laser scanning over a large area means that analysis is performed on an area, not a well basis. This equates to the simultaneous scanning of 4, 16 and 64 wells in 96, 384 and 1536 well format, respectively. Reconfiguration of assays into higher density plate formats results in a concomitant increase in throughput up to 300,000 samples per day in 1536 well microplates.

## 2 Multicolour Cytometric Analysis



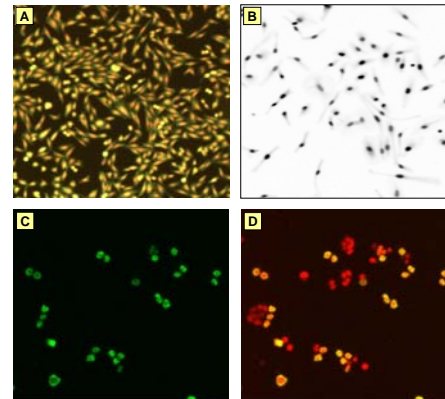
Cytometric data can be represented as two dimensional fluorescence intensity maps (A & B) or 3-dimensional fluorescence intensity profiles (C & D). Multicolour cytometric analysis using an Acumen X3 permits the calculation of fluorescence and morphological parameters for each object identified without image-processing. Data show cytoplasmic staining for tubulin using a FITC conjugate with nuclear counterstaining using propidium iodide.

## 3 Whole Well TIFF Image of Cells Treated With Fluorescent Stain



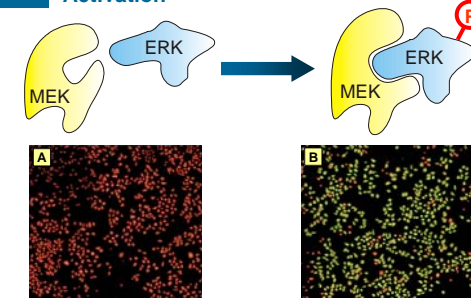
Single whole well TIFF image of THP-1 cells treated with a fluorescent whole cell stain scanned on an Acumen X3. Plate type, 96 well; Scan resolution, 1µm x 1µm. Inset shows a 20x objective equivalent image enlarged from within the well.

## 4 Examples of TIFF Image Export



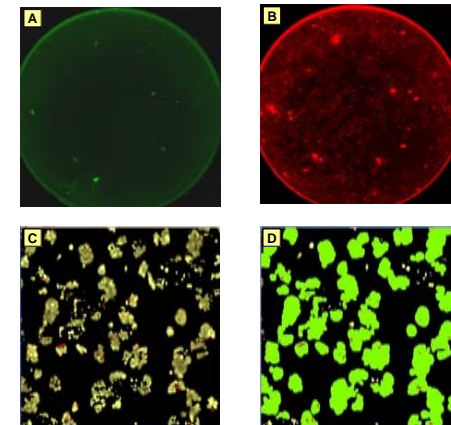
Fluorescently-labelled cells were scanned using an Acumen X3 microplate cytometer and data acquired simultaneously for multiple colours. Scan resolution was 1µm x 1µm and data exported as 8-bit TIFF images. A & B, calcein-AM and propidium iodide (PI). C & D, mitotic index assay using anti-ph3 antibody (FITC secondary) and PI nuclear counterstain. In C, note peripheral localisation of anti-ph3 staining and presence of other cells in telophase. Image processing was performed using Image-Pro Express (Media Cybernetics, Berkshire, UK).

## 5 High Content Analysis of Protein Kinase Activation



Activation of ERK through phosphorylation by its upstream activator MEK in CHO cells. FCS-activated ERK was detected using phospho-p44/42 MAP kinase antibody (CST #9101) and FITC secondary antibody. Cells were counter-stained with propidium iodide. TIFF images from the Acumen X3 software show control well (A) and FCS-treated well (B). The ability of Acumen X3 to analyse the entire well permits normalisation of responses to the total cell number, and compensate for variable cell growth or kinase stimulation within the well.

## 6 Stem Cell Research



Fluorescently-labelled stem cells were scanned using an Acumen X3 microplate cytometer and data acquired simultaneously for multiple colours. A & B, cell proliferation using anti-BrdU antibody (Alexa 488 secondary) and PI nuclear counterstain. Cells in S-phase were quantified using cytometry. C & D, stem cell differentiation using calcein-AM (green) and a selective reporter gene (red). Cytometric data were exported as 8-bit TIFF images and image-processed to identify and count differentiated cells within each cluster (Media Cybernetics, Berkshire, UK). Data were supplied by Epistem Ltd, Manchester, UK.